GeneSpring GT (formerly Varia) Quick Start Guide

The procedures in this guide are for use with Varia Analysis Workbench.

What is Varia Workbench?

Varia workbench provides tools for analyzing genetic variation on a genomic scale. The program can analyze tens or hundreds of thousands of genetic variations simultaneously. The program allows you to:

- View and navigate through a visual representation of all measured variations in human chromosomes
- Perform genetic linkage and association tests to identify relationships between genotypes and phenotypes
- View, calculate, and use haplotypes and haplotype maps
- View and analyze pedigrees
- Study the relationships between genetic variations, genes, and sequence regions

What's New in Varia Workbench A.02.00

- Improved ease-of-use
- New analyses:
  - Non-parametric linkage (NPL)
  - ANOVA
  - Regression
  - Loss of Heterozygosity (LOH)
  - Quantitative Case Control
- The ability to add variations and modify the genome

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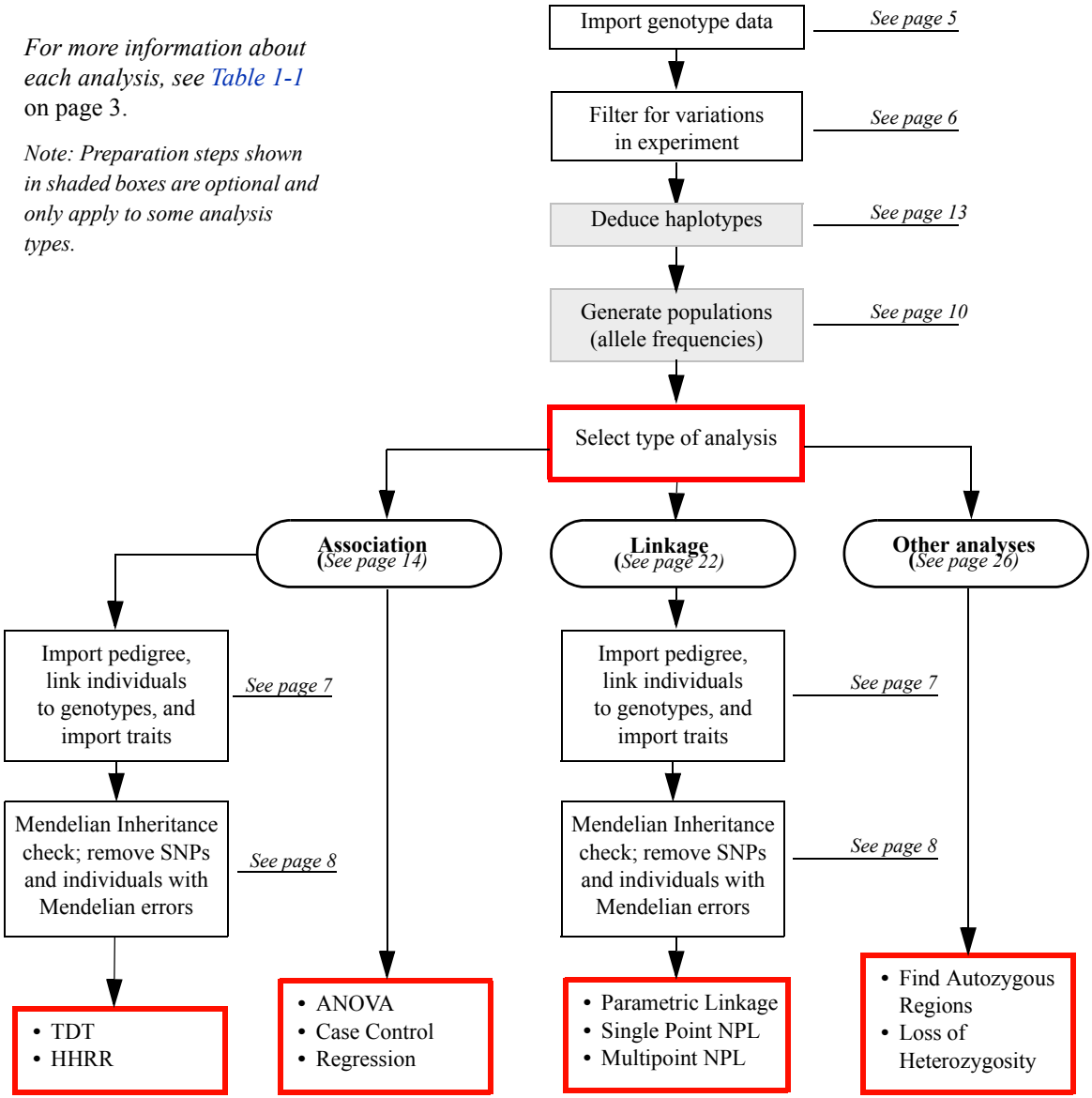
Analyzing Data in Varia Workbench

This diagram shows the activities involved in analyzing data with Varia workbench.

For more information about each analysis, see *Table 1-1* on page 3.

Note: Preparation steps shown in shaded boxes are optional and only apply to some analysis types.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Mendelian Inheritance check; remove SNPs and individuals with Mendelian errors
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Table 1-1 Types of Analyses in Varia Workbench

Analysis Type	Pedigree?	Description
Association		Performed on larger outbred populations. (page 14)
ANOVA (page 14)	No	Finds variations whose genotypes segregate individuals with respect to a quantitative trait. It is used when you have hundreds or thousands of unrelated individuals with high and low values of a quantitative trait.
Qualitative Case Control (page 15)	No	Estimates the linkage disequilibrium between two markers in a group of unrelated individuals with and without a particular phenotype.
Quantitative Case Control (page 15)	No	Estimates the linkage disequilibrium between two markers in a group of unrelated individuals with high and low values of a quantitative trait.
Regression (page 17)	No	Looks for association between genotypes and one or more phenotypes in a large group of individuals (hundreds) when the non-genetic contributions to a disease are thought to be important.
Family-based Association Analysis (TDT and HHRR) (page 19)	Yes	Provides a complete set of linkage disequilibrium statistics that indicate the level of association of a set of variations of interest to a specific trait or affliction Requires a large number of individuals and pedigree data with known genotypes for both parents and their affected offspring.
Linkage		Performed on related groups of individuals. (page 22)
Parametric Linkage (page 22)	Yes	Estimates the recombination frequency between two markers when you have genotyped many people in multiple generations in a pedigree.
Non-parametric Linkage (NPL) (page 24)	Yes	Localizes the genetic basis of a trait when you don't have an exact model of the genetics of the trait, but do know who is affected and unaffected by the disease within a pedigree. A common example of NPL analysis is Affected Sibling Pair analysis (ASP).
Other (page 26)		
Find Autozygous Regions (page 26)	No	Finds homozygous regions in affected individuals that are likely to be inherited from a common ancestor.
Loss of Heterozygosity (LOH) (page 28)	No	Looks for statistically significant regions that are more homozygous in transformed tissues than in pre-transformed tissues or in late-stage compared to early-stage tumors.

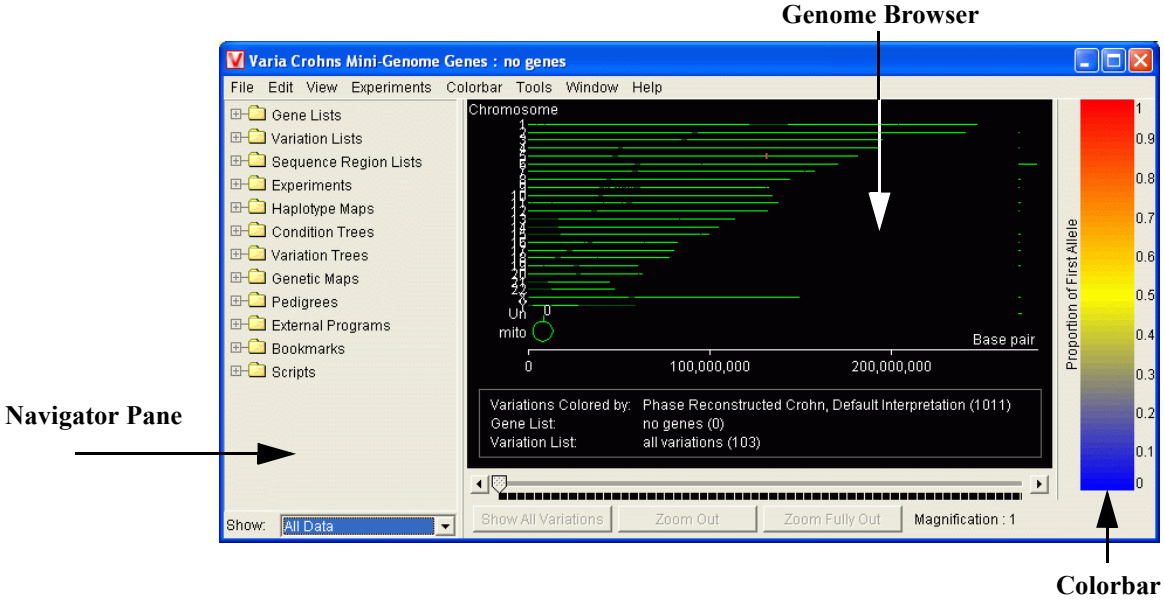
Starting Varia Workbench

1 Install Varia workbench according to the instructions given in the *Varia Workbench Installation Manual*.

2 Double-click the Varia workbench icon on the desktop.

3 A Helpful Hint is displayed that tells you how to load data. Click **OK** to close the Hint window.

The Varia workbench main window appears.



4 Proceed to "Import genotype data" on page 5.

Varia Workbench allows you to analyze data in many different ways. Often the analyses are dictated by the data; how the study was conducted and whether or not you have pedigree information for the individuals in the study.

Preparing for Analysis

The following activities are prerequisites for some analyses in Varia workbench:

Import genotype data

(Applies to all analyses.)

1. Select **Import Data** from the **File** menu.
2. Select a text file that contains the genotype data and click the **Open** button.
3. Confirm the selection of the correct data file format and genome on the **Import Data: Define File Format and Genome** window, then click the **Next** button. In most cases the file format will be Affymetrix or Silicon Genetics Internal SNP.

   Verify that the variations that you are studying are in the current genome build. If not, add your variations to the existing genome prior to importing data by using the **Edit Master Table of Variations** command on the **Edit** menu. See Chapter 16 in the User's Guide for more information.

4. Add additional samples for experiment if needed on the **Selected Files** window, and click the **Next** button.
5. (*optional*) Add attributes on the **Sample Attributes** window. Add any additional information on the individuals in your experiment that is usually in the pedigree file such as gender.

Note The Individual ID is the combined information from family identifier and individual identifier in the pedigree file separated by a period (.). For example, if the first individual in the pedigree comes from family 66 and has the individual identifier 1, the Individual ID will be 66.1. You can add this now or from the Experiment Checklist in Step 9.

6. (*optional*) Add an attribute field such as Founder, if you intend to generate the allele frequencies using only the founders or any individual from your samples. The founder-only method is recommended if you have more than 50 founders from the same population in your sample set (*Ott, 1992*). Mark every individual that is a founder with an uppercase **F** in the appropriate field so that you can sort on it later.
7. To add other attributes, copy the information from your pedigree file and paste it into the respective attribute column. Make sure that the rows in the file you've copied match the **Sample Attribute** window.
8. When you have finished adding attributes, click the **Next** button. You are prompted to **Create Experiment**. Click the **Yes** button to generate the experiment.

9 Name and save the experiment. Use the **New Experiment Checklist** to prepare your data for analysis: **Define Parameters**, **Define the Default Interpretation**, and **Link Pedigree Individuals To Samples**. It is also a good idea to assign a project to the study at this point.

10 (*optional*) Modify attributes as described below.

Modify sample attributes

(Applies to all analyses.)

Use this procedure if you need to modify sample attributes.

1 Select **Sample Manager** from the **Experiments** menu.

2 Click the **Filter on Experiment** tab in the **Sample Manager** window.

3 Select your samples of interest in the **Filter Results** table.

4 Click the **Add** button. The samples appear in the Selected Samples table.

5 Click the **Edit Attributes** button to inspect, change, or add attributes. When you are finished, click **OK** to close the **Edit Attributes** window.

6 Click **OK** to close the **Sample Manager** window.

Filter for variations in experiment

(Applies to all analyses.)

The Varia human genome contains millions of variations. Use the procedure below to create a subset of these variations that contain data specific to your experiment.

1 Select **Find Variations with Data in Experiment** from the **Tools** menu.

2 In the **Run Script** window, select your experiment from the **Experiments** folder in the Navigator pane and click the **Experiment** button in the Inputs area.

3 Click the **Start** button to run the script.

4 **Save** the new variation list, and it will appear in the **Variation Lists** folder in the **Navigator** pane of the Varia workbench main window.

5 (*optional*) Assign a project to the new variation list.

Import pedigree

(Applies to Linkage analyses and Family-based Association analyses only.)

A pedigree describing individuals, their relationships, and affliction status is required to perform family-based genetic analysis. Use the procedure below to import a tab-delimited pedigree text file. After the pedigree has been imported, match individuals in the pedigree to corresponding genotyped individuals and define the traits that are associated with the pedigree.

1. Select **Import New Pedigree** from the **File** menu.
2. Select the pedigree text file that corresponds to your experiment, then click the **Open** button.
3. You are prompted to provide the meaning of any extra columns (traits).
4. Name and save the pedigree. It is also a good idea to assign a project to the pedigree at this point.
5. A **Link Individuals to Samples** window opens automatically. Fill it out now or click **Cancel** to close the window if you don't want to fill it out at this time.

Link individuals to samples

Family-based analyses use genotyped individuals who have been matched to pedigree individuals. When a pedigree is imported, the link window is automatically active, but you can also open the window to make changes as described below.

a Right-click on the pedigree in the **Navigator** pane and select **Inspect** from the shortcut menu to display the **Pedigree Inspector**.

b Click the **Link to Experiment** button on the **Individuals in Pedigree** tab to display the **Link Individuals to Samples** window.

c Link each person from your pedigree to the experimental data. Each person in the pedigree is listed in the **Individuals** table. The persons in the experiment file are listed in the **Samples** table. Persons in the **Individuals** table are automatically linked with persons in the **Samples** table if the **Individual ID** and **Sample ID** match. These pairs are listed in the **Individual/Samples Pairs** table on the right side of the **Link Individuals to Samples** window.

Alternate method You can also add Individual IDs to samples by selecting **Deduce Pedigree** from the **Tools** menu.

Define traits

For some diseases, the inheritance model relating to a pedigree is known. The relevant information such as phenotypes, penetrance, and allele frequencies can be defined for these traits as described below.

a On the **Trait Details** tab of the **Pedigree Inspector** window, click the **Add New** button.

b When the **Edit Trait** window opens, enter the values for the trait, define the disease model, and add penetrance information.

c Click **OK** to close the **Edit Trait** window. The trait you just added appears in the **Trait Details** tab of the **Pedigree Inspector**.

d Repeat Steps a-c for all traits you want to add.

Run Mendelian inheritance check

(Applies to Linkage analyses and Family-based Association analyses only.)

Prior to performing family-based parametric analyses, we recommend evaluating variations for observance of Mendelian inheritance. This procedure will identify genotyping errors.

1. Select **Check Inheritance** from the **Tools** menu.The **Run Script** window opens.
2. Select your experiment in the **Navigator** pane on the left side of the window, and click the **Genotypes** button in the **Inputs** area.
3. Select the **Variation List** for your experiment in the **Navigator** pane, and click the **To check** button in the **Inputs** area. The variation list was created as described in "Filter for variations in experiment" on page 6.
4. Select the **Pedigree** for your experiment in the **Navigator** pane, and click the Pedigree button in the **Inputs** area.
5. Use the supplied default values for the following knobs, or enter different values if you want:
   - Allowable proportions of errors in %
   - P value cutoff, to set what violates the inheritance rules
6. Click the **Start** button. The results are displayed in two tables; one that shows the variations that seem to violate the inheritance rules and one that shows the individuals that violate the inheritance rules.
7. Investigate the variations in the two lists. If necessary, variations or individuals with unusually high genotyping errors can be removed as described below.

Remove individuals with Mendelian errors

Use this procedure after running the Mendelian inheritance check described on page 8.

a (*optional*) Make a copy of your existing pedigree as follows:

- Display the pedigree in **Pedigree Diagram** view in the **Genome Browser** pane of the Varia workbench window.
- Select all the individuals in the pedigree, right-click in the Browser pane, and select **Make Pedigree from Selected Individuals** from the shortcut menu.
- Name and save the copy of the pedigree.

b Using the Check-Inheritance Results table as a guide, remove the trios that have inheritance errors as follows:

- Select the individuals in the Browser pane
- Right-click and select **Delete Selected Individuals** from the shortcut menu.

Note If the parents have another child that does *not* show inheritance errors, then only remove the child that shows errors, rather then the child and both parents.

c (*optional*) Re-run the Mendelian inheritance test to see if the variation list changes.

Remove SNPs with Mendelian errors

Use this procedure after running the Mendelian inheritance check described on page 8.

a Select **Physical Position** from the **View** menu.

b Right-click on the variation list that contains the inheritance errors in **Navigator**, and select **Venn Diagram > Left** from the shortcut menu.

c Right-click on the complete variation list for your experiment in **Navigator** (i.e. the one that includes Mendelian errors), and select **Venn Diagram > Right** from the shortcut menu.

d Select the complete variation list for your experiment again and set as **Universe** using the Venn diagram option. Inspect the Venn diagram on the right side and make sure the lists are correct.

e Right-click on the **non-overlapping** region in the complete variation list (the green segment), and select **Make list of variations in this list only** from the shortcut menu. The new variation list will contain only variations *without* Mendelian errors.

Generate populations to establish allele frequencies

(Applies to some types of Linkage analyses and Autozygosity analyses.)

You can generate populations to establish allele frequencies in the following ways:

- Use your own samples (founders only) (page 10)
- Use Affymetrix frequencies (page 11)
- Import your own allele frequencies (page 11)

Use your own samples (founders only)

The following procedure allows you to generate a population containing allele frequencies using individuals from your experiments.

1 Select **Sample Manager** from the **Experiments** menu.

   a Click the **Filter on Experiment** tab, select the experiment of choice.

   b Sort on attributes (e.g. Founders) in the Filter Results table.

   c Click on each individual in the **Filter Results** table that you want to use to generate allele frequencies. Click the **Add** button to add the samples to the **Selected Samples** table. At least 50 founders should be selected.

   d Click the **Create Experiment** button to create an experiment that contains the individuals in the **Selected Samples** table.

2 Right-click on the sample file in the **Experiment** folder in the **Navigator** pane and select **Inspect** from the shortcut menu.

   a Click the **Interpretation** tab in the **Experiment Inspector** window.

   b Click on **Default Interpretation** and select **Do not Display**.

   c Save this file as a new interpretation (e.g. population condition). This results in a single averaged condition with that interpretation.

3 Run the following script from the **Basic Scripts** folder of **Navigator**:

   Merge-Split Groups > Merge Condition to Sample

   a Select the single averaged condition that you generated in Step 2 from the **Experiment** folder, and click the **Condition** button in the **Inputs** area.

   b Leave the **Data compression** method as **Population**.

   c Click the **Start** button to run the script. A new sample containing population allele frequencies is created and displayed in a **Sample Inspector** window.

   d Name and save the sample. It is also a good idea to assign a project to the sample at this point. This sample is now available in the **Sample Manager** window.

4 (*optional*) Open the **Sample Manager** window from the **Experiments** menu.

   a Sort the samples by date.

   b Add the new population sample to the sample list.

   c Create an experiment from this new sample.

Use Affymetrix frequencies

Affymetrix provides allele frequencies for their SNPs from African-American, Asian and Caucasian populations. Contact Affymetrix for additional information.

1 Drag the Affymetrix **Population Fre.zip** file into the Varia workbench window, or select **Import Varia Zip** from the **File** menu in the main window. Contact Agilent Technical support for information on how to get this file if you don't have it.

2 You can select these population files in the **Experiments** folder in the **Navigator** pane.

Import your own allele frequencies

1 To import your own population frequencies, create an allele frequency file with the following format. This is one of several input file formats supported in the Varia Workbench.

```
# SiGSNP2.0                (Version)
# type=3                   (Type of data: 3=population)
# chroms=2                 (number of Chromosomes in organism)
# samples=1                (number of samples: 1 for populations)
# name=Population          (identity of population)
rs1376173 A:0.2,G:0.8      (SNPID  Allele1:Frequency1,Allele2:Frequency2)
rs1254601 C:0.2,T:0.8      (SNPID  Allele1:Frequency1,Allele2:Frequency2)
rs1254600 C:0.8,T:0.2      (SNPID  Allele1:Frequency1,Allele2:Frequency2)
```

2 Select **Import Data** from the File menu.

a Select the genome, such as **Homo Sapiens build 34v2 db SNP.txt**, and click the **Open** button.

b Click the **Next** button on the **Define File Format and Genome** window.

c Add additional samples for experiment if needed on the **Selected Files** window, and click the **Next** button.

d Click the **Next** button on the **Sample Attributes** window.

e You are prompted to **Create Experiment**. Click the **Yes** button to generate the experiment.

f **Save** the experiment.

Deduce haplotypes

(Applies to Linkage analyses and Family-based Association analyses only.)

Individual variations can be organized as haplotype blocks. The data may be analyzed by variations or haplotype blocks formed by adjacent variations that exhibit linkage. Use the following procedure to generate haplotypes from an experiment if you have pedigree data.

Note If you *don't* have pedigree data, use the script **QC & Analysis > Haplotypes > Deduce Haplotypes (No Pedigree)** in the **Basic Scripts** folder.

Before you start This tool requires that samples have an "Individual ID" attribute. Manually add unique entries in the "Individual ID" attribute field on the **Edit Attributes** window, which you can access from the **Sample Manager** window. Any samples that do not have an "Individual ID" attribute will *not* be included in the resulting pedigree.

1. Select **Deduce Haplotypes** from the **Tools** menu.
2. In the **Run Script** window, select your experiment from the **Experiments** folder in the **Navigator** pane, then click the **Experiment** button.
3. Select the variation list that corresponds to your experiment from the **Variation List** folder in the **Navigator** pane, then click the **Variation List** button.
4. Select the pedigree for your experiment from the **Pedigree** folder in the **Navigator** pane, then click the **Pedigree** button.
5. Accept the defaults provided or enter different values for the following inputs:
   - Error rate
   - Algorithm
   - New Call Confidence
6. Click the **Start** button.
7. You are prompted to create an experiment of the haplotyped samples. Click **Yes** to create an experiment with the samples that contain the computed haplotypes (Haplotype blocks) expression in each (phase-known) individual.
8. Name and save the haplotype experiment and project, if you are using one.

Note If you had defined interpretations in the original non-haplotyped experiment, you will have to redefine them in the haplotyped experiment.

If you have complete family information, the result is a comprehensive list of phase known haplotypes that you can use for Linkage analysis or Family-based association studies (TDT or HHRR).

Association Analyses

Association analyses are performed on larger outbred populations and include the following:

- ANOVA (page 14)
- Case Control (page 15)
- Regression (page 17)

Family-based Association tests (TDT and HHRR) are described on (page 19).

ANOVA

ANOVA (Analysis of Variance) is used to find variations whose genotypes segregate individuals with respect to a quantitative trait. It is used when you have hundreds or thousands of unrelated individuals with high and low values of a quantitative trait.

1 Prepare for ANOVA analysis with the following steps:

   a "Import genotype data" on page 5

   b "Filter for variations in experiment" on page 6

2 Select **Association > ANOVA** from the **Tools** menu.

3 In the **ANOVA** window, select one of the following options for **Estimate the association between a trait** and:

   - Trait
   - Variation
   - Variation List
   - Haplotype Block
   - List of Haplotype Blocks

4 (*Variation or Haplotype block only*) Click the **Find** button to open the **Choose Variation** window, and select the variation of interest.

5 (*Variation List or List of Haplotype Blocks only*) Select the variation list that corresponds to your experiment from the **Variation List** folder in the **Navigator** pane, then click the **Choose Variation List** button.

6 Select your experiment from the **Experiments** folder in the **Navigator** pane, then click the **Choose Experiment** button.

7 (*Haplotype Block or List of Haplotype Blocks only*) Select a haplotype map from the **Haplotype Maps** folder in the **Navigator** pane, then click the **Choose Haplotype Map** button.

8 Select the **Quantitative Trait** of interest from the list.

9 Select one of the following types for **Genetic Variable**:

- **Allele Count** - Divides the samples into groups based on the alleles of the marker locus.
- **Genotype** - Divides the samples into groups based on genotypes of the marker locus.

10 Accept the defaults provided for the **Low** and **High Trait Cutoff** values or enter different values.

11 Click the **Start** button to run the analysis.

12 The results are a statistic for the Trait, Variation and Haplotype Block analysis types and a variation list for the Variation List and List of Haplotype Blocks analysis types. You can save the variation list or copy the table to the Clipboard and paste into Excel.

Case Control

The case control algorithms in Varia workbench are designed to look for measured genotypes that are found more frequently in diseased individuals (cases) than they are in non-diseased individuals (controls). Perform case-control analysis as follows:

1 Prepare for case-control analysis with the following steps:

a "Import genotype data" on page 5

b "Filter for variations in experiment" on page 6

c (optional) "Deduce haplotypes" on page 13

d Check ethnicity to avoid problems with admixture by running the **Personal Genetics > Genetic Admixture** script in the Basic Scripts folder in **Navigator**. See the *Testing for Association* section of the *Varia Analysis Workbench User's Guide* for more information.

2 Select **Association > Qualitative Case Control** or **Association > Quantitative Case Control** from the **Tools** menu, depending on whether the trait you are studying is qualitative or quantitative.

3 In the **Case-Control** window, select the two types of measurements that you want to test for association. These can include almost any combination of the following:

- Traits
- Variations
- Variation Lists
- Haplotype Blocks
- Lists of Haplotype Blocks

For Quantitative Case Control, you select one of the above measurement types to compare to the quantitative trait you select.

4 (*Variation or Haplotype block only*) If you select an individual variation (rather than a list) or an individual haplotype block, look up the identifier for that locus. Click the **Find** button to open the **Choose Variation** window, and select the variation of interest. Note that this option is only available if you have selected a single locus measurement type in Step 3.

5 (*Quantitative case-control only*) Once you define the quantitative trait, you will see a histogram that shows the distribution of values in your experiment. In some studies, statistical power is gained by excluding individuals with mid-level values from the analysis and only using individuals with extreme values of the given trait. Use the diamond-shaped sliders to specify the samples to be used in your experiment, and to identify where the thresholds for high and low values begin. Any samples (values) with a lavender bar beneath them will be included in the experiment.

6 Click the **Start** button to run the analysis.

7 Review and save the analysis results. See the *Varia Analysis Workbench User's Guide* for more information.

   a One of the best ways to see if you have an interesting result is to sort the table based on an interesting statistic, such as the Chi Squared value. Then look at the Physical Position column. If the highest chi squared values are all within a narrow region of the genome, this is an interesting result.

   b Another way to rationalize the results of a case-control analysis is to plot any of the statistics from the resulting variation table in the Physical Position view in the Browser pane. Look for regions with consistently high association scores (or low p-values). If neighboring variations exhibit high association scores, this also indicates that you have found an interesting region.

Regression

Regression analysis is used to identify the contribution of one or more genetic or non-genetic markers to the disease phenotype (dependent variable). This is especially powerful when the non-genetic markers are thought to be influential in their contribution to the disease or are thought to alter the measurement of the disease phenotype. Perform regression analysis as follows:.

1. Prepare for regression analysis with the following steps:
   - **a** "Import genotype data" on page 5
   - **b** "Filter for variations in experiment" on page 6
   - **c** (optional) "Deduce haplotypes" on page 13
   - **d** "Generate populations to establish allele frequencies" on page 10
   - **e** Check ethnicity to avoid problems with admixture by running the **Personal Genetics > Genetic Admixture** script in the Basic Scripts folder in **Navigator**. See the *Testing for Association* section of the *Varia Analysis Workbench User's Guide* for more information.
2. Select **Association > Regression (One Model)** or **Association > Regression (Many Models)** from the **Tools** menu, depending on the type of Regression analysis you want to perform.
   - **One Model** - This mode performs a single regression that can contain any number of genetic or non-genetic covariates.
   - **Many Models** - This mode performs a separate regression (model) for each entry in a list of variations. Each regression is different from the others in that a different independent variable is selected from the variation list. The many models regression can also include any number of genetic or non genetic covariates that are identical for each regression.
3. In the **Regression** window, select one of the following options to **Estimate the association between a trait and a**:
   - Trait
   - Variation
   - Variation List - Note that in the "one model" option, many variation lists are so large that it is unsuitable to include each variation as an independent variable in a single regression.
   - Haplotype Block
   - List of Haplotype Blocks - Note that in the "one model" option, many variation lists (representing haplotype blocks) are so large that it is unsuitable to include each block as an independent variable in a single regression.

4 (*Variation or Haplotype block only*) Click the **Find** button to open the **Choose Variation** window, and select the variation of interest.

5 (*Variation List or List of Haplotype Blocks only*) Select the variation list that corresponds to your experiment from the **Variation List** folder in the **Navigator** pane, then click the **Choose Variation List** button. Note that in the "many model modes" and especially in the "single model" modes, there is a significant statistical penalty for selecting a list that is too long. In general, your results are more likely to be meaningful if you shorten the input list using other criteria (biological or statistical).

6 Select your experiment from the **Experiments** folder in the **Navigator** pane, then click the **Choose Experiment** button.

7 (*Haplotype Block or List of Haplotype Blocks only*) Select a haplotype map from the **Haplotype Maps** folder in the **Navigator** pane, then click the **Choose Haplotype Map** button.

8 Select the **Dependent Trait** of interest from the list.

9 Click the **Choose Variables** button to open the **Choose Independent Variables** window, and select the independent variables to use in the Regression Analysis. These are the non-genetic (or non-dependent) traits that contribute to the disease.

10 Select one of the following types for **Genetic Variable**, which defines the manner in which the genotypes for the Genetic Variable are encoded.

- **Allele Count** - This option includes a single independent variable for each of the possible alleles of the Genetic Variable. For diploid samples, this will always be 0, 1, or 2. This option assumes that the effect of the disease allele is additive.
- **Genotype** - This option includes a set of Boolean variables for (one less than) each of the possible permutations of two alleles. This option assumes that only specific pairs of alleles are associated with the dependent variable.

11 (*Quantitative traits only*) Accept the defaults provided for the **Low** and **High Sample Cutoff** values or enter different values to select the samples to use in the analysis, from the samples already selected in the experiment. You can select samples on the extreme ends of the phenotype, under the assumption that the more extreme the phenotype, the more likely it is to be genetic. Samples are selected by entering values in the **High** and **Low Cutoff** fields or by moving the sliders on the graph. Only samples with quantitative trait values greater than or equal to the high cutoff or less than or equal to the low cutoff are used.

12 Click the **Start** button to run the analysis.

The results are statistics for the Trait, Variation and Haplotype Block analysis types and variation lists for the Variation List and List of Haplotype Blocks analysis types.

13 You can save the variation list or copy the table to the Clipboard and paste into Excel.

Family-based Association (TDT and HHRR)

Varia workbench provides two algorithms for performing family-based association analysis: Transmission/Disequilibrium Test (TDT) and Haplotype-based Haplotype Relative Risk Test (HHRR). These tests are alternative methods of analysis compared to case-control experiments. Both case-control and TDT/HHRR analyses are used to elucidate linkage disequilibrium between an observable trait and a set of variations of interest.

To increase the reliability of the analysis, TDT and HHRR are typically applied to genotype data from at least hundreds of nuclear families with affected children. The analysis requires a large number of individuals and pedigree data with known genotypes for both parents and their affected offspring. The results provide the complete set of linkage disequilibrium statistics that indicate the level of association of a set of variations of interest to a specific trait or affliction.

TDT

Unlike case-control analysis, TDT overcomes the issue of population stratification, a confounding factor resulting from the use of genetically distinct subsets for cases and controls. As a result of population stratification, case-control analysis may reveal significant disequilibrium that result from the inherent difference between the groups, instead of revealing disequilibrium between an affliction and a variation. In TDT, the controls are the parents of affected children. Because of the genetic similarity, controls are appropriately matched to the cases. TDT is also specifically capable to detecting disequilibrium due to linkage.

HHRR

While HHRR is not robust to population stratification, it is an analysis method that is capable of using both homozygous and heterozygous parents, compared to TDT which only includes parents with heterozygous genotypes. HHRR establishes significance of disequilibrium by comparing transmitted to untransmitted alleles to affected individuals.

Perform family-based association analysis as follows:

1 Prepare for TDT/HHRR analysis with the following steps:

   a "Import data and create an experiment" on page 50.

   b "Filter for variations in experiment" on page 56.

   c (optional) "Deduce haplotypes with pedigree data" on page 72.

   d (*HHRR only*) "Check Hardy-Weinberg equilibrium" on page 56.

   e "Import pedigree data" on page 62.

   f "Link pedigree individuals to samples" on page 64.

   g "Define diseases and disease liability" on page 67.

   h "Perform quality control check on pedigree data" on page 65.

   i "Remove individuals with Mendelian errors" on page 66.

   j "Remove SNPs with Mendelian errors" on page 66.

2 Select **Association > Transmission Disequilibrium Test (TDT)** from the **Tools** menu.

3 In the **Transmission Disequilibrium Test** window, select one of the following options to **Estimate the association between a trait and a**:

   - Variation
   - Variation List
   - Haplotype Block
   - List of Haplotype Blocks

4 (*Variation or Haplotype block only*) Enter the identifier of the variation of interest or the variation that corresponds to a haplotype block of interest. If necessary, click the **Find** button to perform a variation search on the **Choose Variation** window.

5 (*Variation List or List of Haplotype Blocks only*) Select the variation list that corresponds to your experiment from the Variation List folder in the **Navigator** pane, then click the **Choose Variation List** button.

6 Select your experiment from the Experiments folder in the **Navigator** pane, then click the **Choose Experiment** button.

   Specify a haplotype reconstructed experiment if you selected **Haplotype Block** or **List of Haplotype Blocks** in Step 3. You can create this experiment by selecting **Deduce Haplotypes** from the **Tools** menu or by selecting **Create New Experiment** from the **Experiments** menu.

7 (*Haplotype Block or List of Haplotype Blocks only*) Select a haplotype map from the Haplotype Maps folder in the **Navigator** pane, then click the **Choose Haplotype Map** button.

8 Select the pedigree for your experiment from the Pedigree folder in the **Navigator** pane, then click the **Choose Pedigree** button.

9 Select the **Trait** of interest from the list.

10 Select **TDT** or **HHRR** as the **Test Type**, depending on which analysis you want to run.

11 Click the **Start** button to run the analysis.

12 Review and save the analysis results.

   For TDT, Chi squared, Degrees of Freedom, and P-value statistics are calculated.

   For HHRR, D, D', Rho, Chi squared, Degrees of Freedom, P-value, and T to U ratio statistics are calculated. T to U is the ratio of the number of times the most highly transmitted allele is transmitted to the number of times it is not transmitted.

   See the *Varia Analysis Workbench User's Guide* for more information.

13 You can obtain a visually informative representation of the data by plotting each disequilibrium statistic from a resulting variation table list in the Physical Position view in the **Browser** pane. Typically you want to look for regions with consistently high association scores (or low p-values). If neighboring variations exhibit high association scores, this is also an indication that you have found an interesting region.

Linkage Analyses

Linkage analyses are performed on related groups of individuals and require pedigree information and include the following analysis options:

- Parametric Linkage (page 22)
- Non-parametric Linkage (NPL) (page 24)

Parametric Linkage

Parametric linkage analysis is used to estimate the recombination frequency between two markers when you have genotyped many people in multiple generations in a pedigree.

1. Prepare for Parametric Linkage analysis with the following steps:
   a. "Import genotype data" on page 5.
   b. "Filter for variations in experiment" on page 6.
   c. (*optional*) Deduce haplotypes as described in "Deduce haplotypes" on page 13.
   d. "Generate populations to establish allele frequencies" on page 10.
   e. "Import pedigree" on page 7, including linking individuals to genotypes, and importing traits.
   f. "Run Mendelian inheritance check" on page 8, including removing SNPs and individuals with Mendelian errors.
2. Select **Linkage > Parametric Linkage** from the **Tools** menu.
3. In the **Parametric Linkage** window, select **Estimate the linkage between Variation List and Trait**.
4. Select the variation list that corresponds to your experiment from the **Variation List** folder in the **Navigator** pane, then click the **Choose Variation List** button.
5. Select your experiment from the **Experiments** folder in the **Navigator** pane, then click the **Choose Experiment** button.
6. Select the pedigree for your experiment from the **Pedigree** folder in the **Navigator** pane, then click the **Choose Pedigree** button.

7 Click the **Choose Population Sample** button to display the **Select Population** window.

   a Select the **Filter on Experiment** tab, then sort the **Filter Results** table by double-clicking the **Type** column heading.

   b Click on the population sample in the **Filter Results** table that contains the allele frequencies generated as described in "Generate populations to establish allele frequencies" on page 10.

   c Click the **Add** button to add it to the **Selected Samples** table.

   d Click **OK** to close the **Select Population** window.

8 Select the **Trait** of interest from the list.

9 Accept the defaults provided or enter different values for the following inputs:

- Theta Values
- Number of Consecutive Variations to Combine

10 Click the **Start** button to run the analysis.

Note The computation can take more than eight hours, depending on the size of your pedigree.

11 When the results are displayed, you can save the resulting table as a variation list or copy the table to the Clipboard and paste into Excel.

Non-parametric Linkage (NPL)

NPL analysis is used to localize the genetic basis of a trait when you don't have an exact model of the genetics of the trait, but do know who is affected and unaffected by the disease within a pedigree. A common example of NPL analysis is Affected Sibling Pair analysis (ASP).

1 Prepare for NPL analysis with the following steps:

   a "Import genotype data" on page 5.

   b "Filter for variations in experiment" on page 6.

   c (*optional*) Deduce haplotypes as described in "Deduce haplotypes" on page 13. This step is important for Multipoint NPL analysis, which assumes that the variations used in the analysis are unrelated to each other, i.e. that they are *not* in linkage disequilibrium. The resulting LOD scores can be artificially inflated if multiple variations from a region with high LD (or "hot zone") are included. In particular, the variation list should be streamlined for Multipoint NPL analysis when using high density chips, so that only one variation represents a region with high LD.

   d "Generate populations to establish allele frequencies" on page 10.

   e "Import pedigree" on page 7, including linking individuals to genotypes, and importing traits.

   f "Run Mendelian inheritance check" on page 8, including removing SNPs and individuals with Mendelian errors.

2 Select **Linkage > Non Parametric Linkage (NPL)** from the **Tools** menu.

3 In the **NPL** window, select **Estimate the association between a trait and a Variation List**.

4 Select the **Single** or **Multiple Point Linkage** option.

5 Select the variation list that corresponds to your experiment from the **Variation List** folder in the **Navigator** pane, then click the **Choose Variation List** button.

6 Select your experiment from the **Experiments** folder in the **Navigator** pane, then click the **Choose Experiment** button.

7 Select the pedigree for your experiment from the **Pedigree** folder in the **Navigator** pane, then click the **Choose Pedigree** button.

8 Click the **Choose Population Sample** button to display the **Select Population** window.

   a Select the **Filter on Experiment** tab, then sort the **Filter Results** table by double-clicking the **Type** column heading.

   b Click on the population sample in the **Filter Results** table that contains the allele frequencies generated as described in "Generate populations to establish allele frequencies" on page 10.

   c Click the **Add** button to add it to the **Selected Samples** table.

   d Click **OK** to close the **Select Population** window.

9 Select the **Trait** of interest from the list.

10 Accept the defaults provided or enter different values for the following inputs:

Single Point Linkage Inputs	Multiple Point Linkage Inputs
• Statistic	• Statistic
• Maximum number of bits for the inheritance vector	• Mapping Function
• Number of Consecutive Variations to Combine	• Error Rate
	• Maximum number of bits for the inheritance vector
	• Include results between variations

11 Click the **Start** button to run the analysis.

Note The computation can take more than eight hours, depending on the size of your pedigree.

12 When the results are displayed, you can save the resulting table as a variation list or copy the table to the Clipboard and paste into Excel.

Other Analyses

In addition to the Linkage and Association analyses described previously, the following additional analyses are available in Varia workbench:

- Find Autozygous Regions (page 26)
- Loss of Heterozygosity (page 28)

Find Autozygous Regions

This analysis finds homozygous regions in affected individuals that are likely to be inherited from a common ancestor. In an inbred population that is enriched in individuals with rare recessive diseases, this approach can be very powerful in identifying regions that span the disease locus. Find autozygous regions as follows:

1. Prepare for to find autozygous regions with the following steps:
   a. "Import genotype data" on page 5
   b. "Filter for variations in experiment" on page 6
2. Select **Find Autozygous Regions** from the **Tools** menu.
3. In the **Find Autozygous Regions** window, select one of the following combinations for the analysis:
   - Individual / Haplotype Map - This option requires a haplotype map for the population that best fits the individual.
   - Individual / Population - This option requires the use of a population sample that describes the allele frequencies of the measured loci. If you do not have a population sample, you can select multiple samples from individuals who you believe are genetically (ethnically) similar to the individual in question.
   - Families / Haplotype Map - This option requires a haplotype map for the population that best fits the individual.
   - Families / Population - This option requires the use of a population sample that describes the allele frequencies of the measured loci. If you do not have a population sample, you can select multiple samples from individuals who you believe are genetically (ethnically) similar to the individual in question.
4. Select the variation list that corresponds to your experiment (or to the variations from your experiment that have passed relevant quality control tests) from the **Variation List** folder in the **Navigator** pane, then click the **Choose Variation List** button.
5. (*Families only*) Select your experiment from the **Experiments** folder in the **Navigator** pane, then click the **Experiment** button.

6 (*Haplotype Map only*) Select a haplotype map from the **Haplotype Maps** folder in the **Navigator** pane, then click the **Haplotype Map** button.

7 (*Families only*) Select the pedigree for your experiment from the **Pedigree** folder in the **Navigator** pane, then click the **Pedigree** button.

8 (*Individual only*) Click the **Choose Individual Sample** button to display the **Select Individual Sample** window.

   a Select the appropriate filtering method, then click on the sample of interest in the **Filter Results** table.

   b Click **OK** to close the **Select Individual Sample** window.

9 (*Population only*) Click the **Choose Population Sample** button to display the **Select Population** window.

   a Select the **Filter on Experiment** tab, then sort the **Filter Results** table by double-clicking the **Type** column heading.

   a Click on the population sample of interest in the **Filter Results** table.

   b Click the **Add** button to add it to the **Selected Samples** table.

   c Click **OK** to close the **Select Population** window.

10 (*Families only*) Select the **Recessive Trait** of interest from the list.

11 Accept the defaults provided or enter different values for the following inputs:

   - Error Rate - The estimated error rate for the genotyping measurement technology. Consult your hardware vendor for good estimates.
   - LOD Score Cutoff - The cutoff of the regional LOD score that is used to build sequence region lists. Segments of sequence with regional LOD scores above the threshold are included in the sequence region lists.

12 Click the **Start** button to run the analysis.

13 When the results are displayed, you can save the resulting table as a variation list or copy the table to the Clipboard and paste into Excel. The analysis also produces a homozygous sequence regions list, which may be saved or copied to the Clipboard.

Loss of Heterozygosity

LOH analysis looks for statistically significant regions that are more homozygous in transformed tissues than in pre-transformed tissues or in late-stage compared to early-stage tumors. Varia workbench allows you to perform LOH studies on groups of tumor/nontumor pairs or on single tumors using a common non-tumor reference. Several summarization methods are available to characterize the most common regions that suffered replication defects. The algorithm is very similar to the one used to detect autozygosity in inbred populations. To use this tool, you need to have pairs of transformed and pre-transformed samples, presumably from the same individuals. You can pair the related samples in either of the following ways:

- If you have used sample attributes to designate individuals and tumor state, select the **Pair By Attributes** option.
- To manually assign the pairs, select the **Assign Custom Pairs** option.

If you have designed your experiment so that instead of sample pairs, you have tumor samples from a number of individuals and a single reference sample representing healthy tissue, you can still use the LOH tool in Varia workbench to analyze the experiment.

Perform the loss of heterozygosity analysis as follows:

1. Prepare for LOH analysis with the following steps:
   a. "Import genotype data" on page 5
   b. "Filter for variations in experiment" on page 6
2. Select **Loss of Heterozygosity** from the **Tools** menu.
3. In the **Loss of Heterozygosity** window, select the variation list that corresponds to your experiment from the **Variation List** folder in the **Navigator** pane, then click the **Choose Variation List** button.
4. Select your experiment from the **Experiments** folder in the **Navigator** pane, then click the **Choose Experiment** button. Optionally, you can use the Add/REmove Samples button add samples or to exclude samples from the experiment.
5. Accept the defaults provided or enter different values for the following inputs:
   - Error Rate - the estimated error rate resulting from the genotyping measurement technology
   - LOD Score Cutoff - - the threshold for the log of odds ratio beyond which a region of variations is considered to have significant loss of heterozygosity
   - Minimum Individuals - the fewest number of individuals that must exhibit LOH for a region to be included in the analysis

6 Pair the pre-transformed and transformed samples either manually (custom pairs) or automatically by attributes. If you select the **Assign Custom Pairs** option, a separate window appears when you click **OK**, which allows you to create the desired pairs of samples (see Step 8).

7 (optional) If you select the **Pair By Attributes** option, click the **Preview** button to see the pairs of Pre-Transformed and Transformed samples that will be used in the analysis. Errors found in any of the samples are displayed at the bottom of the **Preview** window.

8 Click the **Start** button to run the analysis.

   If you selected the **Assign Custom Pairs** option in Step 6, the **Custom Pairs** window appears. Select samples from the **Pre-Transformed** and **Transformed** lists and click the **Add Pair>>** button. The pairs appear in the table on the right side of the window, which is the list of sample pairs that will be used for the analysis. Click **OK** on the **Custom Pairs** window to start the LOH analysis.

9 When the results are displayed, you can save the resulting table as a variation list or copy the table to the Clipboard and paste into Excel. The analysis also produces a sequence regions list, which may be saved or copied to the Clipboard.

What To Do Next

After running the analyses described in the preceding pages, you can use the following procedures to further review the results.

Zoom into regions with high probability of linkage

Use the following procedure when you have a variation list that shows only variations with a high probability of linkage (usually the output from an analysis), and you want to scan for peaks using their associated numbers.

1. Zoom in on a variation in the **Browser**. A graph that shows the associated number, usually an LOD score or P-value, is shown below the variation.
2. Pan the screen right or left with the arrow keys to look at other variations (and their associated graphs) along the chromosome.

Visualize annotated genes

1. Select the genes in the **Genome Browser** in one of the following ways:

Single gene Click on any line or rectangle representing a gene in the **Genome Browser**. The name of the selected gene appears in the legend at the bottom of the **Genome Browser**.

Multiple genes Click on any line or rectangle representing a gene in the **Genome Browser**, then <Shift>-click to select additional genes.

Alternate method <Shift>-click-and-drag across the genes you want to select. A box appears as you drag. When you release the mouse, the selected genes are highlighted (selected).

2. Right-click and select **Make gene list from selected genes** from the shortcut menu.
3. (*optional*) Select **Save Image > Browser** from the **File** menu. This opens the **Export Options** window, which allows you to save the image as a PICT or PNG file for use with other applications.

Menu Commands in Varia Workbench

File	Edit	View	Experiments	Colorbar	Tools
Import Data	Copy	Physical Position	Experiment Inspector	Color by:	Find Variations with Data in Experiment
Open Genome or Array	>Variation List	Tree	Experiment Parameters	Proportion of First Allele	Deduce Pedigree
View Projects	>Gene List	Graph	Experiment Interpretation	Proportion Minus Mean	Check Inheritance
New Window	Paste	Bar Graph	Create New Experiment	Proportion Divided by Mean	Check Hardy-Weinburg Equilibrium
New Linked Window	>Variation List	Scatter Plot	Duplicate Experiment	Estimated Heterozygosity	Deduce Haplotypes
New Pedigree	>Gene List	3D Scatter Plot	Sample Manager	Heterozygosity Divided by Mean	Linkage
Import New Pedigree	Undo	Linkage Disequilibrium (GOLD) Plot		T-Score of First Allele	>Parametric
Import Varia Zip	Find Variation	Pedigree Diagram		T-Test P-Value	>Non-Parametric
Save Bookmark	Find Next Variation	Spreadsheet		Chi Squared Score	Association
Print Image	Advanced Find Variation	Zoom In		Chi Squared P-Value	>ANOVA
Save Image	Inspect Selected Variation	Zoom Out		Cramer's V	>Qualitative Case-Control
Close	Find Gene	Zoom Fully Out		Parameter	>Quantitative Case-Control
Quit	Find Next Gene	Visible		Venn Diagram	>Regression (One Model)
	Advanced Find Gene	Display Options		No Color	>Regression (Many Models)
	Inspect Selected Gene				>Transmission Disequilibrium Test (TDT)
	Find Individual				More Variation Tools
	Search				Find Autozygous Regions
	Filter Navigator				Loss of Heterozygosity
	Standard Attributes				Find Potential Regulatory Sequences
	Edit Master Table of Variations				Create Variation Table
	Preferences				Script Editor

See the *Varia Analysis Workbench User's Guide* for a description of commands that appear on the **Window** and **Help** menus.

Tips for Viewing Data in the Genome Browser

To change the display of data

Mouse Action or Key Stroke	Result	Menu Command
Drag a rectangle across a region that you want to expand. Repeat the process until you reach the desired magnification level.	Zooms in (expands selected region)	**View** > **Zoom In**
Click the **Zoom Out** button at the bottom of the **Genome Browser or right-click** and select **Zoom Out**.	Zooms out one level	**View** > **Zoom Out**
Click the **Zoom Fully Ou**t button at the bottom of the **Genome Browser or right-click** and select **Zoom Fully Out**.	Zooms out completely	**View** > **Zoom Fully Out**
Left arrow, Right arrow	Pans horizontally	None
Up arrow, Down arrow	Pans vertically	None
PageUp, PageDown	Pans vertically by a whole screen	None

To change the Genome Browser view

Select one of the following commands from the **View** menu in the Varia workbench window:

- Physical Position
- Tree
- Graph
- Bar Graph
- Scatter Plot
- 3D Scatter Plot
- Linkage Disequilibrium (GOLD Plot)
- Pedigree Diagram

Note that the Browser view changes automatically when you select certain data objects in the Navigator. For example, when you select a pedigree object, the Browser view automatically changes to Pedigree Diagram view.

To access Browser shortcut menu commands

Right-click in the **Browser** pane to access the following commands:

Menu Command	Result
Zoom Out	Decreases the detail shown in the view
Zoom Fully Out	Returns the view to the original data display
Make List from Selected Variations	Allows you to create a variation list from the selected variations
Make List from Selected Genes	Allows you to create a gene list from the selected genes.
Inspect Selected Variations	Pans vertically
Save Image	Allows you to save the image of the Genome Browser in either a PNG or PICT file format
Save Bookmark	Opens the Save New Bookmark Window. Bookmarks allow you save your current display settings, including experiments, gene list, coloration, and selected genes. The saved bookmark appears in the Bookmarks folder in the Navigator pane, so that you can return to it later.
Delete Sequence Region Element	
Display Options	Opens the Display Options window, which allows you to modify the appearance of the current view.

To select variations and genes

Single variation or gene selection

- Click on any line or rectangle representing a variation or gene in the **Genome Browser**. The name of the selected variation/gene appears in the legend at the bottom of the **Genome Browser**.

Multiple variations or genes selection

1. Click on any line or rectangle representing a variation in the **Genome Browser**.
2. <Shift>-click to select additional variations.

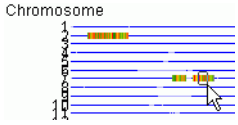
Alternate method

- <Shift>-click-and-drag across the variations you want to select. A box appears as you drag. When you release the mouse, the selected variations are highlighted (selected).
- To *de*select a variation, <Shift>-click on it.

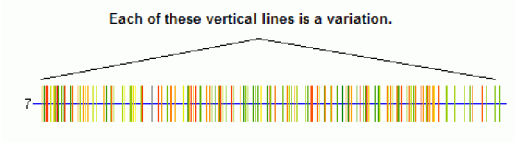
To view data objects

In the Browser area of the Physical Position view, the horizontal lines are chromosomes and the vertical lines are variations.

View variations To zoom in on a chromosome, drag a rectangle to select the area to expand as shown below:

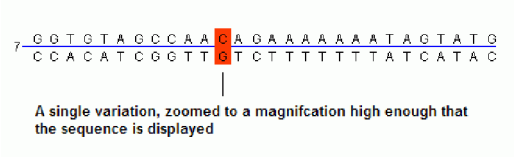


The expanded area is displayed when you release the mouse button, as shown below.

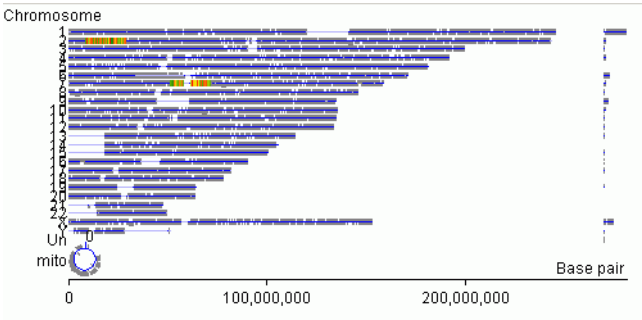


If you repeat this action, you will eventually see the sequence (see *View sequence* below).

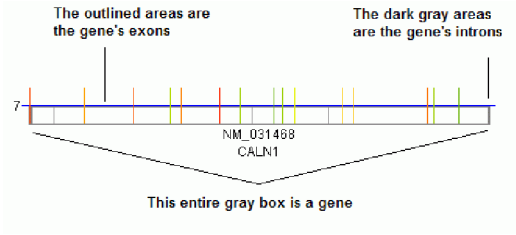
View sequence Continue to zoom in on a chromosome as described in *View variations* above, until the sequence is visible as shown in the example below.


7
G G T G T A G C C A A C A G A A A A A A A T A G T A T G
C C A C A T C G G T T G T C T T T T T T A T C A T A C
A single variation, zoomed to a magnifcation high enough that the sequence is displayed

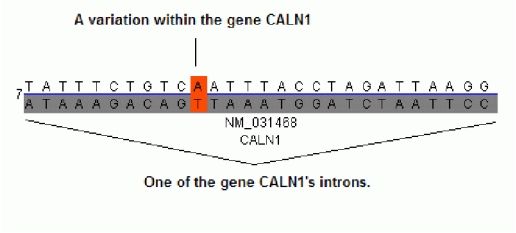
View genes In the Navigator, open the **Gene Lists** folder and click on the **All Genes** list. In Physical Position view, genes appear as many gray boxes on the chromosomes as shown in the example below.



To zoom in on a gene, drag a rectangle to select the area to expand. If you continue to expand the region, the introns and exon regions become visible. Solid color areas in the gene box are coding areas; areas of the gene that are only outlined are exons as shown in the example below:


The outlined areas are the gene's exons
The dark gray areas are the gene's introns
7
NM_031468
CALN1
This entire gray box is a gene

If you expand the area further, the sequence within the gene becomes visible as shown in the example below:

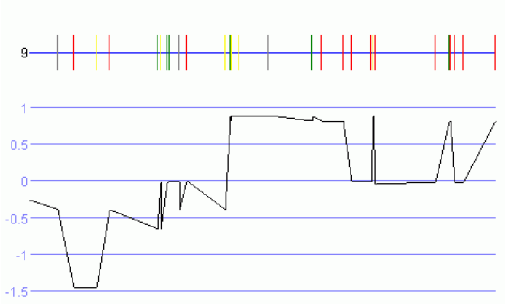

A variation within the gene CALN1
7
T A T T T C T G T C A A T T T A C C T A G A T T A A G G
A T A A A G A C A G T T A A A T G G A T C T A A T T C C
NM_031468
CALN1
One of the gene CALN1's introns.

Display Sequence Regions

In the Navigator, open the **Sequence Region Lists** folder and click on a sequence region. Sequence regions are most often created by running an NPL analysis, as described on page 24. In the Physical Position view, sequence regions appear as many colored boxes on the chromosome. To zoom in on a sequence region, drag a rectangle around it.

Display the numbers associated with a variation list

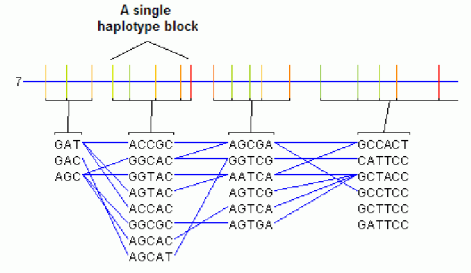
In the Navigator, open the **Variation Lists** folder and click on a variation list that has numbers associated with it. Variation lists that are the results of analyses usually have statistics (numbers) associated with them. You may see no change in the Browser area of Physical Position view if the display is zoomed fully out. Zoom in on a variation and continue to zoom if necessary. A graph is displayed below the variation, with lines that show the number associated with that variation. See example below:



There is a point on the graph for each variation with an associated number in the selected variation list. What the numbers mean depends on the selected variation list.

Display Haplotype Maps

In the Navigator, open the **Haplotype Maps** folder and click on a haplotype map. You may see no change in the Browser area of Physical Position view if the display is zoomed fully out. Zoom in on a variation and continue to zoom if necessary. If the variation is part of a haplotype block, you will see the haplotype block schematic below it as shown in the example below:



The blue lines are the relationships between alleles of neighboring haplotype blocks. In this example, if a person has GAC as the allele for the left most haplotype block, ehtn they are likely to have AGCAT as the allele of the second haplotype block. There is a mouse hover that shows the linkage frequency the line represents.

Where to Find More Information

You can access more information about Agilent Varia workbench as follows.

Agilent Web Site

To view support information for Varia workbench and other Agilent products, see: **http://www.agilent.com/chem/genespring**.

Varia Workbench User's Guide

Consult the *Varia Analysis Workbench User's Guide* either as online help or in printable PDF format for in-depth information about using Varia Workbench. To access online help, click the **Help** button in the Varia Workbench windows or select **User's Guide** from the **Help** menu in the Varia Workbench main window. Table 1-2 tells where to find more information for the topics presented in this Quick Start Guide.

Table 1-2 Where to find more information in the User's Guide

Quick Start Guide	User's Guide How-To	Window or Script
Preparation Steps		
"Starting Varia Workbench" on page 4	Ch. 2, Getting Started	Ch. 1, Introducing Varia Analysis Workbench
"Import genotype data" on page 5	Ch. 2, Getting Started; Ch 18, Master Table of Variations Windows	Ch. 12, Data Import and Experiment Windows
"Filter for variations in experiment" on page 6	Ch. 2, Getting Started	Ch. 21, Basic Scripts
"Import pedigree" on page 7	Ch. 3, Working with Pedigree Data	Ch. 12, Data Import and Experiment Windows
"Run Mendelian inheritance check" on page 8	Ch. 3, Working with Pedigree Data	Ch. 21, Basic Scripts
"Generate populations to establish allele frequencies" on page 10	Ch. 2, Getting Started	Ch. 13, Varia Inspectors and Related Windows
"Deduce haplotypes" on page 13	Ch. 4, Working with Haplotypes	Ch. 21, Basic Scripts

Table 1-2 Where to find more information in the User's Guide

Quick Start Guide	User's Guide How-To	Window or Script
Association Analyses		
ANOVA (page 14)	Ch. 6, Testing for Association	Ch. 14, Association Analysis Windows
Qualitative Case Control (page 15)	Ch. 6, Testing for Association	Ch. 14, Association Analysis Windows
Quantitative Case Control (page 15)	Ch. 6, Testing for Association	Ch. 14, Association Analysis Windows
Regression (page 17)	Ch. 6, Testing for Association	Ch. 14, Association Analysis Windows
Family-based Association (TDT and HHRR) (page 19)	Ch. 6, Testing for Association	Ch. 14, Association Analysis Windows
Linkage Analyses		
Parametric Linkage (page 22)	Ch. 7, Testing for Linkage	Ch. 15, Linkage Analysis Windows
Non-parametric Linkage (NPL) (page 24)	Ch. 7, Testing for Linkage	Ch. 15, Linkage Analysis Windows
Other		
Find Autozygous Regions (page 26)	Ch. 8, Performing Other Analyses	Ch. 16, Other Analysis Windows
Loss of Heterozygosity (LOH) (page 28)	Ch. 8, Performing Other Analyses	Ch. 16, Other Analysis Windows
"Zoom into regions with high probability of linkage" on page 30	Ch. 9, Reviewing Results	Ch. 1, Introducing Varia Analysis Workbench
"Visualize annotated genes" on page 30	Ch. 9, Reviewing Results; Ch. 10, Exporting Varia Data	Ch. 1, Introducing Varia Workbench; Ch. 17, Other Varia Windows
"Tips for Viewing Data in the Genome Browser" on page 32		Ch. 1, Introducing Varia Workbench

Ch. 22, Formulas contains information on formulas used in Varia workbench analyses.